# THE ANTAGONISTIC EFFECT OF STRONTIUM IONS FOR ANESTHETIZATION OF PARAMECIUM CAUDATUM WITH NICKEL IONS

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by

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# CHAPTER I

## INTRODUCTION

The effects of salts of metals upon protozoans has been an object of investigation for many years. Recent studies have been in the area of ion antagonism, and have been concerned with the disparity between the observed experimental results and the theoretically predicted results. Experimental evidence to show ion antagonism between the strontium ion and the nickel ion has not been presented.

Specimens of paramecia may be easily collected in nature, and cultivated in the laboratory. Beginning with a single specimen, one may obtain a rich clone of the animals within a relatively short period of time. The paramecia are microscopic, require relatively little maintenance, and the total space required for large numbers is small. Individual animals may be removed from a drop of culture medium, washed, placed in a test medium, and observed with little expenditure of time.

The animal reproduces asexually by binary fission. The organisms of any one clone are hereditarily alike, insuring the uniformity of the test animals. Long term experiments may be performed upon the same clone of animals. In addition to being a single cell, the paramecium may be considered as an entire organism, and as such carries on all the vital activities of an organism.

The paramecia may be maintained on a standardized diet, enabling one to limit the variables in experimental studies. Paramecia are highly sensitive to the action of toxic substances. It is difficult to devise chemical tests of sensitivity that approach the sensitive reaction of living organisms for toxic agents.

Paramecia have been intensively investigated by many experimenters. The antagonistic effect of the strontium ion is the only one of the alkaline earth elements for which previous studies have not been published. In this study, an attempt has been made to supply additional information on ion antagonism, with particular regard to the strontium ion.

It was the purpose of this investigation (1) to compare the computed effects of strontium ion antagonism

and the observed biological effect of the strontium ion for anesthesia of Paramecium caudatum (Ehrenberg) with the nickel ion; (2) to compare the prolonged effect of the strontium ion antagonism upon the vitality of P. caudatum with control groups.

# CHAPTER II

## REVIEW OF THE LITERATURE

Paramecia have been the object of studies since man has had the optical equipment necessary for their observation. Ehrenberg, on June 11, 1932, as cited by Wichterman,[1] described Paramecium caudatum for the first time. The action of various salts and salt antagonisms upon the ciliary action of P. caudatum (Ehrenberg) has been studied by many; the period from 1900 to 1920 was one of great activity. Wichterman[2] gives a complete review of the literature prior to 1952.

Mast and Nadler[3] reported that monovalent cation salts and hydrates tested (thirty-one), with the exception

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[1] Ralph Wichterman, The Biology of Paramecium. (New York: Blakiston Company, Inc., 1953), p. 7.

[2] Ibid.

[3] S. O. Mast and J. E. Nadler, "Reversal of Ciliary Action in Paramecium caudatum," Journal Morphology and Physiology , XLIII (June, 1926), 117.

of $(NH_4)_2(SO_4)$ and $NH_4C_2H_3O_2$, induced reversal of ciliary action. None of the bivalent or trivalent salts, with the exception of $CaHPO_4$, $MgHPO_4$, and $Ba(OH)_2$ reversed ciliary action. These investigators concluded that the ciliary reversal in paramecia is associated with differential absorption of cations as well as subsequent changes in electrical potential. In addition, other factors were believed to be involved. They further concluded that the action of the cilia are controlled by the neuromotor apparatus.

Eisenberg-Hamburg[1] indicated that P. caudatum recoil from strontium ions, with motion arrested for ten to twenty seconds. This reaction was independent of the anion and characteristic of strontium.

Von Gelei, as cited by P. de Puytorac, C. Andrivon,

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[1]E. Eisenberg-Hamburg, "L'influence des Sels de Strontium sur les Mouvements du Paramecium caudatum. Le Role des Sels de Calcium et de la Concentration en ion Hydrogene", Acta Biologiae Experimentalis, IV, (December, 1930), 262.

F. Serre[1], was the first to state that the nickel ion paralyzed the ciliary apparatus of paramecia, without immediately killing the animal. He used varying concentrations of nickel chloride, nickel sulfate, and nickel nitrate; observing the effect upon the beating of the cilia and the rhythm of the contractile vacuole.

Oliphant[2] stated that reversal of ciliary action was due to, or associated with, an increase in protoplasmic viscosity.

Oliphant[3] also studied the effects of monovalent and bivalent cation salts upon the ciliary action of P. caudatum. His results agreed with Mast and Nadler.[4] He further noted that the effects were due to the cation, not the anion.

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[1]P. de Puytorac, C. Andrivon, F. Serre, "Sur L'action Cytonarcotique des Sels de Nickel Chez Paramecium caudatum (Ehrb.), Journal of Protozoology X (1), (February, 1963), 10.

[2]J. F. Oliphant, "The Effect of Chemicals and Temperature on Reversal in Ciliary Action in Paramecium," Physiological Zoology, XI (January, 1938), 30.

[3]J. F. Oliphant, "Reversal of Ciliary Action in Paramecium Induced by Chemicals," Physiological Zoology, XV (October 1942), 452.

[4]Ibid.

Thomas[1] measured the effects of concentration of nickel sulfate upon the time required for anesthetic action and recovery. To obtain information on the reduction of ciliary movement due to the effect of nickel sulfate, he compared the distance traversed by a normal and an anesthetized paramecium. He reported the rate of movement for normal P. caudatum as 950 microns per second, whereas Sears and Elveback[2] stated that the mean speed of P. caudatum, at twenty-five degrees centigrade, was 400 to 500 microns per second.

Grebecki and Leszek[3] stated that the cation toxicity of chlorides is of the order: Hg > Cu > Ni > Cd > Pb > Zn > Co > Mn > Ba > $NH_4$ > Sr > K > Ca > Mg > Na > Li, and that the toxic action of anions is due chiefly to pH changes.

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[1]Raymond Thomas, "L'action Anaesthesique du Sulfate de Nickel sur Paramecium caudatum," Bulletin de Microscopie Applique, III (September, 1953), 73.

[2]D. F. Sears and L. Elveback, "A Quantitative Study of the Movement of Paramecium caudatum and Paramecium multimicronucleatum," Tulane Studies in Zoology VIII (5), (1961).

[3]Andrzej Grebecki and Kuznicki Leszek, "The Relation of Paramecium caudatum to the Chemical Properties of its Medium and the Protective Effect of Aggregation against Inorganic Substances," Folia Biologiae, III (2), (October, 1955), 157.

More recently, Bovee[1] described the use of nickel sulfate as the ideal anesthetic agent for protozoa.

Lee and McCall[2] found that the pH of medium affected the size of food vacuoles, as well as the length and width of the total animal. Dryl[3] induced ciliary reversal in P. caudatum by simultaneous action of barium and calcium ions.

Yarbrough and O'Kelley[4] found normal swimming of P. caudatum in 0.0002 M. calcium changed to avoidance upon being introduced to equimolar strontium. Equimolar barium had a less pronounced effect.

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[1] Eugene C. Bovee, "Nickel Sulfate as an Anesthetic for Protozoans," Turtox News, XXXVI (2), (February, 1958), 78.

[2] Warren J. Lee and William McCall, "Effects of pH and Viscosity on Surface Membranes in Paramecium multimicronucleatum," Journal of Protozoology, VI (2), (May, 1959), 149.

[3] Stanislaw Dryl, "The Ciliary Reversal in Paramecium caudatum Induced by Simultaneous Action of Barium and Calcium Ions," Journal of Protozoology, VIII (Suppl.) (December, 1961), 16.

[4] James D. Yarbrough and Joseph C. O'Kelley, "Alkaline Earth Elements and the Avoidance Reaction in Paramecium multimicronucleatum, Journal of Protozoology, IX (2) (May, 1962), 135.

Jahn[1], in his studies of ion antagonism and ciliary reversal, analyzed ciliary reversal in terms of the Gibbs Donnan ratio. He proposed that a given bond angle produces a specific ionic effect. This introduced a new concept of stereochemistry concerning the biological effects of ions.

Leeuwenhoek, in 1676, as cited by Dobell[2], was the first to observe cilia, and recognize their use in locomotion. Many investigators have studied the detailed structure of the cilia. Peter, as cited by Wichterman[3], demonstrated the fact that the cilia on the fragments of a ciliate will continue to beat if they are in connection with a piece of cytoplasm. Gelei and Klein, as cited by Wichterman[4], studied the cilia by fixation and stained or impregnated preparations. Their accounts differ, probably due to their inability to clearly

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[1]Theodore Louis Jahn, "The Mechanism of Ciliary Movement II Ion Antagonism and Ciliary Reversal," Journal Cellular and Comparative Physiology, LX (3) (October, 1962), 228.

[2]Clifford Dobell, Antony Van Leeuwenhoek and His Little Animals (New York: Russell and Russell Inc., 1958), p. 127.

[3]Ralph Wichterman, The Biology of Paramecium (New York: Blakiston Company Inc., 1953), p. 64.

[4]Ibid., p. 50.

see the minute structures with the available optical equipment. Kudo[1] describes the action of the cilia as the preparatory and effective stroke.

Jakus and Hall[2] made electron microscope observations of cilia using a shadow-casting technique with chromium. This showed a cillium of paramecium to consist of a bundle of eleven fibers. The bundle of dried fibers was between 300 and 500 A. in diameter. Most of the recent work in the study of ciliary morphology has been with the use of electron microscopy.

According to Wichterman[3], Maupas was the first to use the rate of fission as an index of the vitality of the organism. Calkins[4] states that any environmental condition which affects any one link of the vital activities has an

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[1]R. R. Kudo, Protozoology (third edition; Springfield, Illinois: C. C. Thomas, 1947), p. 111.

[2]M. A. Jakus and C. E. Hall, "Electron Microscope Observations of the Trichocysts and Cilia of Paramecium," Biology Bulletin, XCI (October, 1946), 144.

[3]Ibid., p. 361.

[4]Gary Nathan Calkins, The Biology of the Protozoa (Philadelphia: Lea and Febiger, 1933), p. 1.

effect upon the general activity, and that viability is the sum total of all protoplasmic activities set up in response to stimuli.

Several investigators have studied the effect of nickel sulfate upon the rate of reproduction of P. caudatum. A recent study is that of P. de Puytorac, C. Andrion, and F. Serre[1], who found that the fission rate was reduced for approximately twenty generations after the paramecia were immobilized by three grams per liter of nickel sulfate.

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[1] Ibid., p. 18.

# CHAPTER III

## MATERIALS AND METHODS

The paramecia used in the experiment were collected from a small pond within the city limits of Charles City, Iowa. The location of the pond was R-15W, T-96N, Floyd County, Niles Township, the north-west one fourth of section six. With the aid of several texts,[1,2,3] the organism was identified as Paramecium caudatum Ehrb.

From this sample, paramecia were removed and cultivated upon a timothy hay tea and Aerobacter aerogenes media. From the dense population of paramecia established by this procedure, two paramecia were isolated. The individual paramecium was washed within depression slides in seven successive baths of sterile distilled water. Transfer of the paramecium was accomplished with a micro

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[1] Theodore Louis Jahn, How to Know the Protozoa (Dubuque: Wm. C. Brown Co., 1949).

[2] R. R. Kudo, Protozoology (third edition; Springfield: C. C. Thomas, 1957).

[3] Ralph Wichterman, The Biology of Paramecium (New York: Blakiston Company Inc., 1953).

pipet. The paramecium was then placed in a depression slide, a few drops of nutrient medium was added, and the concavity sealed with a cover slip and petroleum jelly. The specimen and medium were then observed at twenty-four hour intervals to note the viability of the paramecium, and to inspect for other contaminating micro-organisms. Species pure clones one and two were isolated and used throughout the experiment. To raise large populations of the two clones, specimens were transferred to glass containers containing one liter of the hay infusion medium. The cultures were maintained in rooms where they were not subjected to direct sunlight. Temperatures ranged from twenty-five to thirty degrees centigrade.

Preparation of the medium was as follows: leaves, stems, and heads of timothy hay (Phleum pratense) were chopped into one to two centimeter sections. (All of the hay was from one stand to keep the mineral content uniform.) Three grams of the chopped hay were added to one liter of tap water from the Charles City water system. (See Table I in appendix.) The medium was then autoclaved (in a Presto cooker-canner, National Presto Industries Inc.) for ten minutes at fifteen pounds of pressure. Before use, the

medium was inoculated with a twenty-four hour culture of Aerobacter aerogenes (furnished by Dr. Rodney A. Rogers, Drake University). P. caudatum was then introduced into the medium about twenty-four hours later. Once a week, some of the paramecia were transferred to a new culture medium. This was done to keep the waste products, which are toxic, from becoming too concentrated. Paramecia used in this experiment were from cultures that were from one to three weeks old. With the exception of some experimental trials with starved paramecia, all the paramecia used in the experiment were from flourishing, well-nourished clones.

Distilled water (the distilled water used during this experiment was doubly distilled in pyrex glass), hydrated nickel chloride, $NiCl_2.6H_2O$ and the hydrated strontium chloride, $SrCl_2.6H_2O$, (chemicals obtained from Wm. Welch Scientific Company) were used to prepare stock solutions of $8.4 \times 10^{-3}$ molality. All weight measurements were made on a Voland Company 200 B chain analytical balance (Wm. Welch Scientific Company). Further dilutions were made by volumetric measurements from the original stock solution. The concentration of nickel and strontium chloride in the

solution used in the experimental work was $8.4 \times 10^{-4}$ molal unless otherwise indicated.

The experimental technique for observation of anesthesia was as follows: Paramecia were transferred by pipet to a depression slide. The individual paramecium was then washed in three successive baths of glass distilled water, and placed within the depression of a ground glass slide with a micro pipet. The pipets were calibrated, as nearly as possible, to give thirty drops to the milliliter. To the drop containing the paramecium was added a drop of solution containing the ions in the concentration to be tested. For low power observation and for handling the organisms, a Bausch and Lomb "zoom" steroscopic microscope with a magnification of 10x to 20x was used. For detailed observation of ciliary movement, functioning of the contractile vacuole, and bleb formation, high dry and oil immersion lens on various compound microscopes were used.

To observe with higher magnification, a drop of the ion solution containing a paramecium was transferred to a glass depression slide and a cover slip placed thereon. This was then viewed under a high dry or oil immersion lens.

To retain a sample for prolonged observation, a ring of petroleum jelly was placed upon the slide, a drop of the ion solution containing a paramecium was placed upon the cover slip, the slide inverted and pressed lightly against the cover slip. This procedure sealed the chamber and enabled one to observe a particular specimen for ten to twelve hours without the organism being destroyed by evaporation.

For this experiment, death of the organism was determined by three criteria: cessation of all movement of the cilia, complete stoppage of contractile vacuole activity, and disintegration of the cell body. Injuries and effects of a less serious nature were determined by observing: changes in ciliary activity or locomotion, changes in rate of cyclosis, changes in volume of the cell body, changes in rate of anterior and posterior contractile vacuole activity, trichocyst extrusion, formation of "blebs" or blisters by extrusion or rupture of the pellicle, and disarrangement of internal organization. The above criteria was adapted from Wichterman.[1]

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[1]Ralph Wichterman, The Biology of Paramecium (New York: Blakiston Company, Inc., 1953), p. 430.

Anesthesia was considered to have occurred when all forward locomotion of the organism had ceased, and the cilia were beating with an abnormal stroke and recovery in an unsynchronized manner.

To study the prolonged effects upon the paramecium's vitality, observation was made of the life cycle, using isolation cultures and the fission rate as an index of the vitality as reported by Calkins.[1]

Two types of vitality studies were made. The first consisted of observing the fission rate for a twenty-four hour period, the second was a prolonged study to note the fission rate over a five-day period. The method of recording the fission rate was modified from that of Eckert and Feiler as cited by Wichterman.[2] The method used consisted of recording a number for the number of divisions in a twenty-four hour period. For instance, if four paramecia were found in a depression where one had been isolated, the result

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[1]Gary N. Calkins and Francis M. Summers, Protozoa in Biological Research (New York: Columbia University Press, 1941), p. 527.

[2]Ibid., p. 362.

would indicate two divisions, and be so recorded. Twelve paramecia would indicate three and one-fourth divisions, etc.

The procedure as used consisted of isolating a paramecium and washing it three times in distilled water. The paramecium was then placed within one-tenth milliliter of distilled water within a glass spot plate depression. The test solution was then introduced in a one-tenth milliliter aliquot. At the end of two minutes, the paramecium was removed, again washed three times in distilled water, examined for injury, and then placed within the depression in a glass spot plate. One-tenth of a milliliter of hay infusion medium was added to the fluid transferred with the paramecium. When the nine depressions in the glass spot plate were similarily filled, the plate was covered to prevent evaporation. The paramecia were then observed twenty-four hours later, when a count was made to determine the fission rate.

The prolonged study consisted of duplicating the above procedure, but at the end of each twenty-four hour period, one individual from each depression plate was isolated and placed within one-tenth milliliter of nutrient

medium. The cycle was repeated every twenty-four hours for five-day periods. The glassware used in the experiment was initially autoclaved for ten minutes at fifteen pounds pressure. Thereafter, it was rinsed three times in hot water and dried with paper toweling. The pH of the medium ranged from 6.7 to 7.2 for all experiments.

Measurements of the time required for anesthesia were made with a stop watch, or a watch with a "sweep" second hand. The mean time for anesthesia and standard deviation were calculated for each clone and the various ion solutions. The mean rate of fission and standard deviation were calculated for each clone. Comparison of the mean rate of fission of the control organisms and those treated with the test solutions were made to find the effect of the test solution upon the organism's vitality.

The average dimensions of the P. caudatum of clone one were: length; 252.5 microns, width; 58.4 microns. For clone two: length; 217.8 microns, width, 49.5 microns. This was for fifty individuals.

# CHAPTER IV

## RESULTS AND INTERPRETATION OF DATA

The paramecia of clones one and two were experimentally tested for the time necessary for anesthetization with $NiCl_2.6H_2O$ and for $NiCl_2.6H_2O$ antagonized with an equimolal concentration of $SrCl_2.6H_2O$. For anesthetization, those paramecia anesthetized with $NiCl_2.6H_2O$ were considered the control organism. Introduction of a drop of stock $8.4 \times 10^{-4}$ molal solution into a drop of distilled water containing a paramecium lowered the actual concentration of nickel ions to $4.2 \times 10^{-4}$ molal. The typical reaction sequence of paramecia introduced into a $4.2 \times 10^{-4}$ molal solution of nickel ions was: avoidance, loss of synchronization of ciliary beat, ineffective stroke and recovery of the cilia, reduction in rate of locomotion, swelling (probably due to the reduced rate of operation of the contractile vacuole), anesthesia, discharge of trichocyst, bleb formation or blistering, and death.

The mean time for anesthesia was but slightly different for clones one and two. (See Table II.) The

TABLE II

THE TIME REQUIRED FOR ANESTHESIA OF Paramecium caudatum WITH $4.2 \times 10^{-4}$ MOLAL SOLUTIONS OF NICKEL AND STRONTIUM CHLORIDE

| Solution | Calculated mean time of anesthesia | Observed mean time of anesthesia (in seconds) | Standard deviation (in seconds) |
|---|---|---|---|
| $NiCl_2.6H_2O$ | | Clone 1 39.7 | 16.4 |
| $NiCl_2.6H_2O$ | | Clone 2 40.5 | 29.1 |
| $NiCl_2.6H_2O$ + $SrCl_2.6H_2O$ | 50.5 seconds | Clone 1 84.20 | 33.7 |
| $NiCl_2.6H_2O$ + $SrCl_2.6H_2O$ | 51.3 seconds | Clone 2 74.80 | 23.5 |

For fifty individuals

concentration of nickel ions employed was highly toxic to the paramecia. Death usually resulted within a few minutes.

Paramecia introduced into a $4.2 \times 10^{-4}$ molal solution of $SrCl_2.6H_2O$ reacted in a manner that was typical for strontium ions. Described as "recoil," by Eisenberg-Hamburg[1], this reaction was manifested by the test organism swimming backwards for a few seconds, probably indicating ciliary reversal. The concentration was not toxic to the organism, nor did it have any discernible anesthetic effect.

Paramecia in a drop of distilled water, to which a drop of $8.4 \times 10^{-4}$ molal $NiCl_2.6H_2O$ and $SrCl_2.6H_2O$ was added, exhibited a response unique for that solution. The sequence was as follows: avoidance, a to and fro movement (apparently a backward sweep with the cilia moving the organism forward, followed by a forward sweep of the cilia moving the organism backward. The net effect was a jerky motion of the organism with little forward movement.), slight swelling (probably

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[1]E. Eisenberg-Hamburg, "L'influence des Sels de Strontium sur les Mouvements due Paramecium caudatum. Le role des Sels de Calcium et de la Concentration en ion Hydrogene," Acta Biologiae Experimentalis, IV (November, 1930), 261.

due to reduction in the rate of the contractile vacuole activity), and anesthesia. At that concentration of nickel and strontium ions, blebs were rarely formed. The concentration was not lethal for most of the organisms, during the observation period.

To calculate the theoretical time for anesthesia with $4.2 \times 10^{-4}$ molal solution of nickel ions antagonized by equimolal strontium ions, use was made of the Denye-Huckel expression, as cited by Daniels and Alberty.[1]

$$-\ln \gamma_i = \frac{e^3 z_i^2}{(DkT)^{3/2}} \sqrt{\frac{2\pi N \mu}{1000}}$$

where $\gamma_i$ = activity coefficient of ion species i.
$z_i$ = charge on ions species i.
e = charge of an electron = $4.803 \times 10^{-10}$ electrostatic unit.
D = dielectric constant of the solution = 78.56 for water at 298 K.
N = Avogadro's number = $6.023 \times 10^{23}$.
k = gas constant per molecule = R/N = $1.3805 \times 10^{-16}$ erg degree$^{-1}$ molecule$^{-1}$.
$\mu$ = ionic strength = $\frac{1}{2}(c_1 z_1^2 + c_2 z_2^2 + c_3 z_3^2 + \ldots)$, the summation being taken over all the ions in the solution, where $c_i$ is the concentration of ion species i in moles per liter.
T = absolute temperature.

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[1] Farrington, Daniels and Robert A. Alberty, Physical Chemistry (New York: John Wiley and Sons, Inc., 1955), p. 484.

Introducing the mean activity coefficient $\gamma$, and putting in numerical values for water at twenty-five degrees centigrade, for an electrolyte containing three kinds of ions, the equation becomes $-\log\gamma = 0.509\, z_1 z_2 z_3 \sqrt{\mu}$.

Substituting in this equation the molality of $NiCl_2.6H_2O = 4.2 \times 10^{-4}$, $SrCl_2.6H_2O = 4.2 \times 10^{-4}$, charge of nickel ion = 2, charge of strontium ion = 2, charge of chloride ion = 1. The equation becomes:

$$-\log\gamma = \frac{(0.509)\ (2)\ (2)\ (1)}{\sqrt{\tfrac{1}{2}\left[(4.2 \times 10^{-4} \times 4)+(4.2 \times 10^{-4} \times 4)+(8.4 \times 10^{-4} \times 2)\right]}}$$

$$-\log\gamma = 0.1022012$$

$$\gamma = 0.79$$

Thus, 79% of the nickel ions are active in the solution. Using this figure, one may calculate the theoretical time for anesthesia, e.g., letting 40.50 seconds represent the time necessary for anesthesia in a solution containing nickel ions at 100% activity, a solution containing 79% of the nickel ions active should theoretically require 51.3 seconds to anesthetize the organism. (See Table II, p. 21.)

Isolation cultures of P. caudatum clones one and two were made. The fission rate of the control organism, maintained on hay infusion medium, was used as an index of vitality. The mean rate of fission and the mean rate of reproduction of the control organism was used as the standard in comparison with the test organisms. (See Tables III and IV.) Clones one and two treated with a two-minute immersion in $4.2 \times 10^{-4}$ molal solution of the test ions, then washed and transferred to hay infusion medium, gave the following results. (See Table V.)

Data obtained from anesthetization of P. caudatum with nickel ions antagonized by equimolal strontium ions, showed that there was a biologically inhibitory effect exhibited by the strontium ions for nickel ions.

Although extreme care was taken to be accurate in all measurements, it must be noted that even the slightest variation in the drop size would greatly alter the concentration of ions in solution. Variation in drop size could result from chipping of the aperture of the glass pipet, shaking of the operator's hand, temperature differentials, differences in solution concentration,

TABLE III

THE TWENTY-FOUR HOUR FISSION RATE OF *Paramecium caudatum* TREATED FOR TWO MINUTES WITH NICKEL AND STRONTIUM CHLORIDE SOLUTIONS

| Concentration of solution | Time immersed in solution | Clone | Mean X | S. D. |
|---|---|---|---|---|
| Control | | Clone 1 | 1.86 | 1.09 |
| | | Clone 2 | 1.69 | 1.27 |
| $4.2 \times 10^{-4}$ molal $NiCl_2.6H_2O$ | 2 min. | Clone 1 | 0.79 | 0.39 |
| | 2 min. | Clone 2 | 0.72 | 0.46 |
| $4.2 \times 10^{-4}$ molal $NiCl_2.6H_2O$ + $SrCl_2.6H_2O$ | 2 min. | Clone 1 | 1.35 | 0.84 |
| | 2 min. | Clone 2 | 1.20 | 0.78 |

For fifty individuals

TABLE IV

THE TWENTY-FOUR HOUR POPULATION GROWTH OF *Paramecium caudatum* TREATED WITH NICKEL AND STRONTIUM CHLORIDE SOLUTIONS

| Concentration of solution | Time immersed in solution | | Range | Mean X | S. D. |
|---|---|---|---|---|---|
| Control | | Clone 1 | 1-10 | 3.55 | 2.18 |
| | | Clone 2 | 1-9 | 3.58 | 2.53 |
| $4.2 \times 10^{-4}$ Molal $NiCl_2.6H_2O$ | 2 min. | Clone 1 | 1-4 | 1.40 | 0.67 |
| | 2 min. | Clone 2 | 1-6 | 1.44 | 0.91 |
| $4.2 \times 10^{-4}$ Molal $NiCl_2.6H_2O$ + $SrCl_2.6H_2O$ | 2 min. | Clone 1 | 1-8 | 2.62 | 1.69 |
| | 2 min. | Clone 2 | 1-8 | 2.41 | 1.55 |

For fifty individuals

TABLE V

REPRODUCTION AND FISSION RATE OF *Paramecium caudatum*, TREATED FOR TWO MINUTES IN $4.2 \times 10^{-4}$ MOLAL SOLUTIONS OF NICKEL AND NICKEL ANTAGONIZED BY STRONTIUM IONS, AS COMPARED WITH THE CONTROL ORGANISM

| Reproduction rate | Control | $Ni^{++}$ | $Ni^{++}/Sr^{++}$ |
|---|---|---|---|
| Clone 1 | 100% | 39% | 74% |
| Clone 2 | 100% | 40% | 67% |

| Fission rate | Control | $Ni^{++}$ | $Ni^{++}/Sr^{++}$ |
|---|---|---|---|
| Clone 1 | 100% | 42% | 73% |
| Clone 2 | 100% | 43% | 71% |

For fifty individuals

differences in vapor and barometric pressure of the air, angle at which the pipet was inclined, etc., making the volumetric measurements of drops the least accurate measurement of this experiment.

The physical differences of the test organisms vary to some degree. Clone two showed a rather large S. D. for anesthesia with nickel ions. (See Table II, p. 21.) Two individuals from Clone two digressed markedly from the mean, anesthetization time. One was in the 198-202 seconds range, and one was in the 103-107 seconds range. In Clone one, for anesthesia with nickel ions antagonized by strontium ions, four individuals are within the 208-292 seconds range.

These digressions may be due to experimental error or to individual variation. A limited number of tests were run on paramecia immediately after they had undergone fission. To the drop of medium containing the organisms, a drop of the test ion solution was added. Often there would be a variation in the time for anesthesia to occur, the most extreme variation being nine minutes for a pair of organisms within a $4.2 \times 10^{-4}$ molal nickel and strontium solution.

With those variations in mind, it is interesting to note that the anesthetic effects of the ion solutions upon the clones were very similar.

On three occasions during the vitality studies, "double-monsters" were noted in Clone one control medium. Attempts were made to establish a pure clone of these individuals, but none reproduced. Why these individuals occurred is not known. Wichterman[1] cited Balbiani, Calkins, Peebles, and Alverdes as having observed monsters that occurred after cutting and regeneration of paramecium. Wichterman[2] cited Herzfeld as having been able to produce double-monsters by exposing P. caudatum to hard, chlorinated tap water. Wichterman[3] cited Lloyd as finding monsters occurring in cultures exposed to one part per ten million of benzene hexachloride "gammexane," a compound widely used

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[1]Ralph Wichterman, The Biology of Paramecium (New York: Blakiston Company Inc., 1953), p. 355.

[2]Ibid.

[3]Ibid., p. 358.

as an insecticide. It is believed that the paramecia of Clone one were exposed to "malathion," an insecticide used in the laboratory where the investigation was carried out. It would be interesting to further study the effect of this compound to see if it could be responsible for the abnormalities noted.

With the larger volumes of medium and test solutions utilized in the vitality studies, a more quantitative measurement was obtained. The results of the vitality studies show a more normal standard deviation.

P. de Puytorac, C. Andrivon, and F. Serre[1] state that the metallic salt toxicity is a relation between the cation and the lipids and proteins, with the nickel ion being one of the more toxic cations. With weak doses of the nickel ion, the lesions are reversible. This takes about twenty generations to complete. In the vitality series of this experiment, the paramecia were not observed for a long enough period to confirm their observation. Paramecia treated with

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[1]P. de Puytorac, C. Andrivon, and F. Serre, "Sur L'action Cytonarcotique des Sels de Nickel Chez Paramecium caudatum Ehrb," Journal of Protozoology, X (February, 1963), 17.

nickel ions antagonized by strontium ions had recovered normal rate of fission at the end of five days. However should be pointed out that the fission rate of the two c employed in the experiment was slightly lower than that paramecia observed by other investigators as cited by Ca and Summers.[1]

It should also be noted that in experiments of comparison of the action of salts of different dilutions or of one salt of different dilutions, to the action of different salts, the operation should always contain the same number of organisms in the same physical state. According to Grebecki and Kuznicki,[2] there is a protecti effect of aggregation against inorganic substances. Thi protection is due to the cations in solution being bounc anions within the cytoplasm of the organisms. Death of

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[1]Gary N. Calkins and Francis N. Summers (editors) Protozoa in Biological Research (New York: Columbia University Press, 1941), p. 528.

[2]Andrzej Grebecki and Leszek Kuznicki, "The Relation of Paramecium caudatum to the Chemical Propert of its Medium and the Protective Effect of Aggregation Against Inorganic Substances," trans. A. Pigon, Folia Biologica, III (October, 1955), 127.

individual organism does not immediately release the cati to the environment.

Oliphant[1] demonstrated that ciliary reversal occur immediately with all monovalent alkaline earth ions, with $Ba^{++}$ and $Mn^{++}$, but not with other non-toxic divalent cati ($Sr^{++}$ and $Mg^{++}$). E. Eisenberg-Hamburg[2] stated that in solutions non-fatal to P. caudatum, there is a reaction o recoil specific for $SrCl_2$.

It was observed that the normal movements of the paramecia were interrupted for ten to twenty seconds, immediately after contact with strontium ions. In this experiment, it was further noted that P. caudatum usually exhibited an avoidance reaction which often consisted of swimming backwards, indicating ciliary reversal.

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[1] Joseph Oliphant, "Reversal of Ciliary Action in Paramecium Induced by Chemicals," Physiological Zoology, XV (October, 1942), 452.

[2] E. Eisenberg-Hamburg, "L'influence des Sels de Strontium sur les Mouvements du Paramecium caudatum. Le role des Sels de Calcium et de la Concentration en ion Hydrogene," Acta Biologiae Experimentalis, IV (December, 1930), 261.

Jahn[1] raised the question of the role of the dival ion within the cell membrane, and gives two possible explanations, one morphological and one physiological: ( to maintain the mechanical structure of the cell membrane and (2) to provide an electron conductor for reactions in cell membrane. One might conjecture that the more active strontium ion replaces the calcium ion within the cell membrane, but is not as readily replaced by the nickel io thereby decreasing the toxic effect of the nickel ion. Confirmation of this hypothesis must await future studies

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[1]Theodore Louis Jahn, "The Mechanism of Ciliary Movement. II Ion Antagonism and Ciliary Reversal," Journal of Cellular and Comparative Physiology, LX (October, 1962), 223.

# CHAPTER V

## SUMMARY

The present study considered the effects of strontium ion antagonism for the nickel ion upon anesthesia of Paramecium caudatum and upon their recovery from anesthesia using the fission rate as an index of vitality.

The time required for anesthesia when the organism was placed within a $4.2 \times 10^{-4}$ molal solution of the test ions was observed and recorded. Using the Debye and Huckel expression, the theoretical time for anesthesia with nickel ions antagonized by equimolal strontium ions was computed. Comparison of the observed time for anesthesia with nickel ions antagonized by strontium ions and the theoretical time showed that there was a 67% increase in the time required for anesthesia of Clone one, and a 46% increase in the time required for anesthesia of Clone two. This demonstrated an antagonistic effect of the strontium ions for nickel ions.

Comparison of the mean rate of fission in vitality studies of P. caudatum treated, for two minutes, with $4.2 \times 10^{-4}$ molal solutions of nickel ions antagonized with

equimolal strontium ions showed that Clone one had a mea
fission rate 71% greater, and Clone two 67% greater than
those clones treated with nickel ions alone.

It was evident from the above results, that the
strontium ion was biologically antagonistic to nickel io
However, the mode of action and site of this biological
activity must await further study.

# BIBLIOGRAPHY

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## A. BOOKS


Calkins, Gary Nathan. The Biology of the Protozoa. Se edition. Philadelphia: Lea and Febiger, 1932.

Calkins, Gary N., and Francis N. Summers (editors). Protozoa in Biological Research. New York: Columb University Press, 1941.

Daniels, Farrington, and Robert A. Alberty. Physical Chemistry. New York: John Wiley and Sons, Inc., 1

Dobell, Clifford. Antony Van Leeuwenhoek and His Litt Animals. New York: Russell and Russell Inc., 1958

Giese, Arthur C. Cell Physiology. Philadelphia: W. B Saunders Company, 1962.

Heilbrunn, L. V. An Outline of General Physiology. Philadelphia: W. B. Saunders Company, 1956.

Jahn, Theodore Louis, and Frances Floed Jahn. How to Know the Protozoa. Dubuque: Wm. C. Brown Company, 1949.

Kudo, R. R. Protozoology. Third edition. Springfiel Illinois: C. C. Thomas, 1947.

Prosser, C. L., F. A. Brown, et al. Comparative Anima Physiology. Philadelphia: W. B. Saunders Company, 1951.

Wichterman, Ralph. The Biology of Paramecium. New Yo Blakiston Company, Inc., 1953.

## B. PERIODICALS

Bovee, Eugene C. "Nickel Sulfate as an Anesthetic for Protozoans," Turtox News, XXXVI (February, 1958), 78.

Dryl, Stanislaw. "The Ciliary Reversal in Paramecium caudatum Induced by Simultaneous Action of Barium and Calcium Ions." Journal of Protozoology, VIII (December, 1961), 16.

Eisenberg-Hamburg, E. "L'influence des Sels de Strontium sur les Mouvements due Paramecium caudatum. Le role des Sels de Calcium et de la Concentration en ion Hydrogene." Acta Biologiae Experimentalis, IV (December 1930), 261-277.

Grebecki, Andrzej, and Leszek Kuznicki. "The Relation of Paramecium caudatum to the Chemical Properties of its Medium and the Protective Effect of Aggregation Against Inorganic Substances," Trans. A. Pigon. Folia Biologic III (October, 1955), 127-157.

Jahn, Theodore Louis. "The Mechanism of Ciliary Movement. II Ion Antagonism and Ciliary Reversal," Journal of Cellular and Comparative Physiology, LX (October, 1962) 217-228.

Jakus, M. A. and C. E. Hall. "Electron Microscope Observation of the Trichocysts and Cilia of Paramecium, Biological Bulletin, XCI (October, 1946), 141-144.

Johnson, Willis H. "Further Studies on the Sterile Culture of Paramecium," Physiological Zoology, XXV (January, 1952), 10-15.

Lee, J. Warren and William McCall. "Effects of pH and Viscosity on Surface Membranes in Paramecium multi-micronucleatum," Journal of Protozoology, VI (May, 1959), 146-149.

Marsland, D. A. "Quieting Paramecia for the Elementary Student," Science, XCVIII (November, 1943), 414.

Mast, S. O. "The Food Vacuole in Paramecium," Biological Bulletin, (February, 1947), 31-71.

Moment, G. B. "A Simple Method for Quieting Paramecia and Other Small Organisms During Prolonged Observations," Science, XCIX (June, 1944), 544.

Oliphant, Joseph F. "Effect of Chemicals and Temperature on Reversal in Ciliary Action in Paramecium," Physiological Zoology, XI (January, 1938), 19-30.

_____. "Reversal of Ciliary Action in Paramecium Induced by Chemicals," Anatomical Record, XXCI (December, 1941), 67.

_____. "Reversal of Ciliary Action in Paramecium Induced by Chemicals," Physiological Zoology, XV (October, 1942), 443-452.

Puytorac, P. de, C. Andrivon, and F. Serre. "Sur L'action Cytonarcotique des Sels de Nickel Chez Paramecium caudatum Ehrb," Journal of Protozoology, X (February, 1963), 10-19.

Stanley, John. "A Method of Growing Dense Cultures of Paramecium," Science, CIII (January, 1946), 115-116.

Thomas, Raymond. "L'action Anaesthesique du Sulfate de Nickel sur Paramecium caudatum," Bulletin de Microscopie Applique, III (September, 1953), 73-76.

Wang, H. "Analyses of a Toxic Factor, Lethal to Paramecium, Present in Non-glass Distilled Water," Proceedings Society of Experimental Biology and Medicine, CI (August, 1959), 682-686.

Harbrough, James D., and Joseph C. O'Kelley. "Alkaline Earth Elements and Their Avoidance Reaction in Paramecium multimicronucleatum," Journal of Protozoology, IX (May, 1962), 132-135.

## C. LEARNED SOCIETY

Ehert, C. F., and E. L. Powers. "The Cell Surface of Paramecium," International Review of Cytology, VIII pp. 97-133. New York: New York Academic Press, 1959.

## D. MISCELLANEOUS

Sears, D. F., and L. Elveback. "A Quantitative Study of the Movement of Paramecium caudatum and Paramecium multimicronucleatum," Tulane Studies of Zoology, VIII (1961), 127-139.

## E. UNPUBLISHED MATERIALS

Houser, P. J., "Analysis of Charles City Public Water Supply," 1960 (Personal communication).

# APPENDIX

TABLE I

ANALYSIS OF CHARLES CITY
PUBLIC WATER SUPPLY

STATE HYGIENIC LABORATORY[1]
June 14, 1960

| Well number | | 1 | 3 | 4 | 5 | 6 | Aver. |
|---|---|---|---|---|---|---|---|
| Depth (feet) | | 1530 | 1350 | 1320 | 185 | 1550 | |
| Minerals | | (All results as Mgs/L) | | | | | |
| Calcium as | Ca | 70.4 | 65.6 | 62.0 | 60.4 | 57.2 | 63.00 |
| Potassium as | K | 5.8 | 3.7 | 6.3 | 1.5 | 2.8 | 4.02 |
| Sodium as | Na | 13.7 | 8.9 | 11.2 | 3.7 | 6.8 | 8.86 |
| Chlorine as | Cl | 7.0 | 1.0 | 1.0 | 0.5 | 1.0 | 2.10 |
| Bicarbonate as | $HCO_3$ | 283.0 | 288.0 | 283.0 | 249.0 | 278.0 | 276.20 |
| Silica as | $SiO_2$ | 9.8 | 10.4 | 8.4 | 13.2 | 11.6 | 10.68 |
| Iron as | Fe | 22.0 | 38.0 | 101.0 | 150.0 | 106.0 | 83.40 |
| Magnesium as | Mg | 19.4 | 19.4 | 22.6 | 14.8 | 21.6 | 19.56 |
| Total hardness as | $CaCO_3$ | 256.0 | 244.0 | 248.0 | 212.0 | 232.0 | 238.40 |
| pH | | 7.45 | 7.45 | 7.5 | 7.45 | 7.5 | 7.47 |

(Well #2 not in use)

[1]P. J. Houser, Director, Public Health Engineering, Iowa State Department of Health, Personal communication.